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ON THE BEHAVIOR OF AMEBA TOWARD FRAGMENTS OF GLASS AND CARBON AND OTHER INDIGESTIBLE SUBSTANCES, AND TOWARD SOME VERY SOLUBLE SUBSTANCES.

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INTRODUCTION.

The experiments recorded in this paper were carried out in order to come a step closer to understanding the nature of the stimulus which, emanating from an insoluble particle such as ovalbumin, zein, or lactalbumin, results in an ameba moving

toward that particle. As pointed out in a previous paper (Schaeffer, '16b) in which the reactions of ameba toward proteins are recorded, these isolated proteins, although insoluble enough to satisfy the practical chemist that they may be classed as 'insoluble,' may nevertheless undergo an inappreciable amount of solution sufficient to stimulate an ameba's sense organs. There is however no direct means of knowing that such solution takes place; it is nothing more than a possibility. It has seemed practicable therefore to let the investigation of the behavior toward isolated *insoluble* proteins stand for the present, and to test the reactions of ameba toward some of the purest and most insoluble substances known to determine whether it is necessary that a substance be soluble in order that an ameba may sense it at a distance. In addition to these tests, a number of experiments were also made with very soluble substances and with solutions.

The main conclusions of this paper are based on the reactions toward carbon, glass, tyrosin and peptone. A sufficient number of experiments with these substances are described and figured, I trust, to illustrate, if not to prove, the conclusions. But in addition to these, one or more typical experiments with each one of various other substances have been figured to support the conclusion that the behavior toward the substances specified above is not directly dependent upon the chemical nature of the substances, but upon their more generalized physical properties.

Reactions to Chemically Insoluble Indigestible Substances.

Carbon.—This substance was prepared as follows. India ink in stick form was boiled in xylol until the xylol remained clear. The residue was washed in chloroform and then boiled in sulphuric acid, then filtered and washed with distilled water. The residue was then boiled in potassium hydroxide solution. After acidifying, the carbon was filtered and washed with distilled water acidified with hydrochloric acid. The carbon was then dried and heated to redness for fifteen minutes in a closed platinum crucible. This method of purification should have removed all the soluble constituents present with the carbon in the india ink.

Although carbon as thus prepared is quite insoluble, it is not inert, for carbon has the property of adsorbing certain gases from the surrounding medium. In order to render this action as ineffective as possible on the sense organs of the ameba, the carbon grains, which it will be remembered were in all cases microscopic, were immersed in the water in which the ameba was, thirty minutes before being brought near the ameba. It is believed that this period of immersion permitted the adsorptive and diffusive processes to come as near as is possible to an equilibrium.

A grain of pure carbon was placed in the path of a granular[1] ameba (Fig. 42, Plate I.). As the ameba moved forward it turned to the right, but after passing the carbon it turned to the left. After coming into contact with the carbon a pseudopod was thrown out on the right through which the ameba moved away. The same piece of carbon was again laid before the ameba—47—but the behavior was indefinite.

Several experiments were made on a granular ameba from another culture. A grain of carbon which was laid in its path produced a mild positive reaction: the partial encircling of the carbon—183–188. The carbon grain was then shifted but the commotion caused by moving it led the ameba to react negatively. The carbon was shifted again, producing finally a mild positive reaction. When the carbon was shifted again—189—the ameba turned to the right. As it passed by the carbon, at about twenty microns, two little pseudopods were sent out in the region of the carbon, one of which was headed directly toward the carbon. The ameba moved directly into contact with the carbon, and then moved on through that pseudopod—193.

In the path of another granular ameba was placed a grain of carbon—250. The ameba moved on straight forward, passing the carbon on the right. When the ameba was about half past the carbon numerous pseudopods began to make their appearance on both sides of the ameba, before the ameba had come into

[1] See my paper ('16) pp. 533–536, where the granular and raptorial forms of amebas are described. After this paper was in manuscript the specific identity of the amebas was specially examined. The 'granular' amebas were of two species: *Amœba proteus* and *A. discoides*. The 'raptorial' were of the species *A. dubia*. See my paper in Science, '16, vol. 44, pp. 468–469.

contact with the carbon. The ameba came into contact with the carbon later but the direction of movement was not on that account changed. Although the stimulus from the carbon grain was localized yet the resulting change in behavior involved the whole ameba, for it broke up into a number of pseudopods of which those on the right side of the ameba had of course no direct relation whatever with the carbon grain. The *meaning* of these pseudopods on the right is obscure, though pseudopods are frequently formed in this way under such conditions as surrounded this experiment. Orderly movement in clavate form was disturbed by the sensing of the carbon grain, but after the carbon grain was left behind, orderly movement was again resumed. It should be noted especially that a pseudopod was formed on the right side directly opposite the carbon. This phenomenon is frequently observed in ameban behavior. A number of cases are described in a previous paper (Schaeffer, '16). See also Fig. 224 in this paper. Throughout the whole experiment the general direction of motion was not changed, but it could not be predicted from Fig. 259 in what direction the ameba was then going to move. This experiment is a very good example of the phenomenon of functional inertia (Schaeffer, '12, '14)—the tendency in an ameba to continue moving in the direction in which it started to move—about which I shall have more to say later.

A grain of carbon was placed in the path of a raptorial (see footnote, p. 305) ameba—216. The ameba moved into contact with the carbon, then forked and moved on through the right prong. The carbon grain was then shifted so that it lay to the right of the ameba's path—220. The ameba moved forward, turning away from the carbon at first, but later a side pseudopod was sent out directly toward it. When the ameba was about forty microns from the carbon another pseudopod, further anterior, was also sent out toward the carbon. The posterior pseudopod was the first one to come into contact with the carbon, and as it continued moving forward, it pushed the carbon grain along. The anterior pseudopod moved into contact with the carbon. A pseudopod directly opposite the anterior one enlarged and carried the ameba away. Both pseudopods extended toward

the carbon were retracted as the ameba moved away through the pseudopod which had been forming on the opposite side. Some time later another grain of carbon was laid in the ameba's path—226. Two pseudopods were formed on the right, through the anterior one of which the ameba moved away, disturbed probably by the commotion caused by placing the carbon in the ameba's path. The same piece of carbon was again laid ahead of the ameba—230. The ameba at first turned away from the carbon—231, 232—but later turned toward it—233. A pseudopod was then sent out toward the carbon until it came into contact with the carbon—235–237, then it was retracted and the ameba moved off, directly away from the carbon—239. A few minutes later a new piece of carbon was laid in the ameba's path some distance ahead—240. The ameba then broke up into several pseudopods, but finally moved ahead a short distance through the main pseudopod. A pseudopod was then sent out on the right, which moved into contact with the test substance and apparently ingested it in a typical food cup. The ameba kept on moving forward, and two minutes after apparent ingestion the carbon was left behind. (The curved leader line straightened out, is the measure of the distance the ameba moved away in a straight course from the carbon grain.) The same piece of carbon was again laid ahead of the ameba—264. As the ameba moved forward it turned to the left and away from the carbon, but as it passed by the carbon two pseudopods were sent out toward it—267. The posterior one came into contact with the carbon, moved on over it, and spread out; but no attempt at ingestion was made. The pseudopod forked and the ameba moved along the left prong. A new piece of carbon was then laid to the left of the ameba's path—271. As the ameba moved forward, a pair of side pseudopods which were begun simultaneously on opposite sides—274—continued to enlarge until the one on the left began to flow over the carbon. Then the pseudopod on the right was retracted and the ameba flowed away through the pseudopod extended over the carbon.

To summarize the behavior toward carbon: The most striking feature of the behavior toward pure carbon is that the ameba can sense this substance at a distance of at least forty microns. It

is of course not surprising that soluble objects should be thus sensed, but the sensing of an absolutely insoluble substance at a distance is unique among eyeless animals. It is possible that the carbon grains acted as permanent centers of diffusion of gases adsorbed previously, or adsorption of gases dissolved in the water, and so may have produced differences in their distribution in the water. If these differences in distribution of gases are assumed to come within sensing range of the ameba, then one could understand the observed behavior. I believe however that the gas adsorptive qualities of carbon do not in themselves constitute the stimuli to which ameba reacts when it comes near the carbon; for glass, which is not supposed to adsorb gases to the same extent as carbon, stimulates ameba in a similar manner and quite as markedly.

Practically all of the behavior toward carbon is positive. The negative behavior observed was due to the commotion produced by placing the carbon in position. In most cases the pseudopod which was sent out to the source of the stimulus, was retracted after it had come into contact with the carbon, but in some cases the ameba flowed on through such an exploring pseudopod. Only in one case was ingestion attempted. That it is a real case of partial ingestion is shown by the fact that the process was incomplete; for if the initial stimulus had come from an unobserved small flagellate, for example, on the carbon grain, it is fairly certain that the ingesting process would have been completed. It is reasonable to suppose that the stimuli causing partial ingestion came from the carbon grain.

Both granular and raptorial amebas react to carbon at a distance. The raptorial seem to be attracted somewhat more strongly than the granular.

Glass.—Although glass is a complex substance and is very slightly soluble, neither of these properties by themselves play a part in the stimuli received by amebas; for the fragments of glass were taken, in nearly every case, from the dish or slide on which they and the amebas were later placed in experimenting. The effect of its solubility may therefore be thought to have been cancelled physiologically by the solubility of the glass surface on which it lay.

The glass dishes and the fragments were all carefully cleaned before using. The glass fragments were powdered in a glass mortar and then washed. The culture fluid was carefully filtered, and the amebas were transferred through several washes of filtered culture solution.

A fragment of glass was placed near a raptorial ameba—125. As the main pseudopod moved forward it forked, the left limb moving toward the glass—126, 127. The right pseudopod moved on for a short distance, then turned sharply toward the left and moved into contact with the glass—128–130. The right limb was then withdrawn and the ameba moved off through the left. The glass seems to have been sensed at a distance of about forty microns—128, 129—perhaps at sixty microns—126. A few minutes later a new fragment of glass was laid in the ameba's path—133. The ameba at first turned to the right, but a little later a pseudopod was sent out on the left which moved almost into contact with the glass, but was then withdrawn—136–139. The main pseudopod broke up into three pseudopods, one of which moved a short distance toward the glass and was then retracted. The pseudopod on the right, which with the left one already mentioned formed a pair of opposite pseudopods, then became the main pseudopod through which the ameba moved away. There is no doubt that the ameba received stimuli proceeding from the glass; the formation of the pair of opposite pseudopods shows it, as does also the retraction of the left pseudopod before the glass was reached. The same piece of glass was again laid in the ameba's path—141. The ameba moved toward it a short distance, then turned slightly to the right and moved on—142–144. When the tip of the main pseudopod was even with the glass fragment, a side pseudopod was sent out toward the glass and into contact with it—145, 146. The tip of the main pseudopod also turned over toward the glass and then moved into contact with it. Both pseudopods were then withdrawn while the ameba moved off through a pseudopod on the right. The ameba sensed the glass in Fig. 142 at a distance of over sixty microns. The same piece of glass was then shifted —149. The ameba moved directly forward to within about forty microns of the glass, when the tip of the pseudopod spread

out and later forked and flowed on under the glass fragment. The same piece of glass was again laid before the ameba, but the behavior does not show definitely that the glass was sensed at a distance. The same piece of glass was then shifted and again laid to the left of the ameba's path—160. The ameba moved forward a short distance, then sent out a pseudopod on the left directly toward the glass—162. The pseudopod was called forth doubtless by the agitation of the needle in placing the glass in position, for hungry raptorial amebas are readily thus stimulated. But the glass was actually sensed in Figs. 163 and 164, for the pseudopod directed slightly to the right of the glass turned so as to go directly toward the glass. After coming into contact with the glass a pseudopod was formed on the convex side of the main pseudopod, a region especially favorable to the formation of new pseudopods, through which the ameba moved away. A new piece of glass was then laid before the ameba—168. A pseudopod was thrown out on the right through which the ameba moved on—169–171. This pseudopod turned strongly to the left toward the glass. When about eighty microns from the glass—172—a pair of opposite pseudopods were formed near the tip of the main pseudopod. The left member of this pair of pseudopods moved directly toward the glass. When almost in contact with the glass this pseudopod was retracted—173. The ameba moved away through the main pseudopod. The same piece of glass was again laid before the ameba—175. The ameba moved toward it a short distance, when a pair of opposite pseudopods were formed near the tip of the main pseudopod—177, 178. As the ameba moved past the glass, another pair of opposite pseudopods were formed near the tip of the main pseudopod—178. Neither of these pseudopods moved far before they were retracted—179, 180. Before the ameba moved out of sensing distance of the glass, it turned strongly to the left and encircled the glass through 180° at a distance of about sixty microns. Two pseudopods, soon to be retracted, were formed on the convex side of the ameba during the latter stage of the encircling reaction—181, 182.

A small fragment of glass was laid in the path of a granular ameba—194. The ameba broke up into several pseudopods, of

which the left member of the middle pair became the main pseudopod. As this one moved forward with the glass particle on its right, a pseudopod which appeared on its right, enlarged and moved directly into contact with the glass—197, 198. When the pseudopod came into contact with the glass, streaming became more rapid and the ameba flowed over the glass particle and moved away.

To summarize: The behavior of ameba toward glass fragments, under the conditions above outlined, demonstrates even more clearly than the reactions toward pure carbon, that insoluble objects can be sensed at a distance. The maximum distance at which glass can be sensed, as demonstrated by experiment, is about sixty microns, though it is probable that in several of the experiments the amebas sensed the glass particles at 100 microns. The ameba does not always react positively when glass is sensed, but positive behavior is much more frequent than negative. Although the ameba starts moving toward the glass particle in almost all cases, it sometimes reverses the direction of motion when almost in contact with it. In most cases however the ameba continues moving until it comes into contact with the glass, and then the behavior becomes more or less indifferent. No attempt was made to eat particles of glass.

Graphite.—A grain of Merck's purified graphite was laid in the path of a granular ameba—202. The ameba turned to the right and moved directly toward the graphite until it came within about fifteen microns of the object, when protoplasmic streaming was interrupted for an instant and then directed upwards and away from the graphite—207–209. The piece of graphite was then shifted—210. The ameba turned to the right and away from the graphite, but a pseudopod which was then sent out on the convex side elongated and became the main pseudopod until it came into contact with the graphite (the contact stage is not figured)—214, 215. It was then slowly retracted while the previous main pseudopod became active again and led the ameba away. The precision of the reaction indicates that the graphite was sensed at a distance of at least sixty microns.

The effect of graphite on the reactions of ameba is similar to that produced by glass. Graphite usually produces a positive

reaction. In no case was ingestion attempted. The solubility of graphite was not tested by me.

Silicic Acid.—Merck's Pure, by Wet Process. A small grain of silicic acid was laid in the path of a granular ameba—309. The ameba moved forward a short distance—within about forty microns of the silicic acid—then swelled up at the anterior end and finally sent out a pseudopod on the left through which the ameba moved off. A new grain of silicic acid was then encountered—314. The ameba moved directly into contact with it at the side. The ameba then moved off through a pseudopod sent out posterior to the test object—319–321. A new grain of silicic acid was then laid in the ameba's path—322. After moving toward it a short distance—until within about thirty-five microns of the acids—323, 324—the ameba moved away through a pseudopod thrown out on the left. Another new grain of silicic acid was then laid in front of the ameba—328. The ameba moved forward to within sixty-five microns of the acid, then moved off through a pseudopod thrown out on the left.

Silicic acid is sensed at a distance like carbon, glass and graphite, but with the ameba used in the experiments recorded above, the behavior was nearly always negative. Owing to incomplete knowledge concerning the purity of this substance, it is not clear what the meaning is of the preponderance of negative behavior in the reactions of this ameba. The negative tendency cannot have been due to lack of hunger however for a grain of globulin was readily eaten a few minutes later.

Hematin.—Merck's, according to Nencki. This compound results from the decomposition of hæmoglobin, and is very rich in iron. The black "melanin" produced by the malarial organism is supposed to be hematin, and is said to have a toxic effect on the human body. The following experiment is typical of the behavior of ameba toward hematin. A grain of hematin was placed in the path of a granular ameba—28. As the ameba passed it on the right at a distance of about forty microns, several small pseudopods were sent out but none of them came into contact with the hematin.

Hematin seems to call forth about the same behavior as glass or carbon; perhaps the positive reactions are not quite so decided.

Hematin is not toxic to the ameba; when eaten with food materials it may remain in the ameba for many hours without producing any ill effect.

Indigotin.—Merck's reagent, indigo blue. To the left of the path of a granular ameba was placed a grain of indigotin—278. The ameba moved forward until the tip of the main pseudopod was past the test object—279. A pseudopod was then sent out to the left, which moved into contact with it—280, 281. As the ameba moved forward, it moved out of contact with the indigotin. A small pseudopod which was sent out into contact with the indigotin from the mid-region of the ameba—283—remained in contact with the test substance for a few minutes while the ameba moved on, but it was finally pulled away.

Ameba reacts more decidedly positively to indigotin than to hematin, glass, or carbon. In no case however was ingestion attempted.

Cholesterin.—Eimer and Amend's. In the path of an active granular ameba with many pseudopods, was placed a grain of cholesterin—35. As the ameba moved forward two pseudopods were sent out toward the cholesterin—37—but only one of them came into contact with it. The ameba moved off through a pseudopod thrown out on the right, leaving the cholesterin behind.

As far as my experiments go, it appears that cholesterin belongs in the same class as carbon, glass, etc. No attempt at ingestion is made. Cholesterin is sensed at a distance of at least fifty microns.

Starch Grains from Arrowroot.—Taylor's Commercial. A grain of arrowroot starch was placed in the path of a three-pronged raptorial ameba—288. The ameba moved forward through the middle pseudopod directly into contact with the starch grain and then passed on, on the right, after forming and retracting two pseudopods on the left. The ameba then happened to move toward another mass of arrowroot starch—294. When the ameba came within about thirty microns of the starch, it withdrew from the starch and moved forward to the right—296—but the pseudopod lying nearer the starch became the main pseudopod—297. When the tip of the main pseudopod

had passed beyond the starch—298—two side pseudopods were thrown out toward the starch—299—the anterior one of which came into contact with it—300. Both the side pseudopods were retracted as the ameba started to flow away through the vestige of a former pseudopod shown in Fig. 296 with the arrow. While moving forward the ameba passed another mass of starch grains without reaction. A grain of globulin was then ingested but was excreted a few minutes later.

Arrowroot starch grains are sensed at a distance and usually induce positive behavior. The reactions are more decidedly positive than those induced by glass or carbon, but no attempt at ingestion was observed. A number of experiments with cornstarch produced essentially the same results as those with arrowroot starch.

Lead Oxide.—Eimer and Amend's Pure Yellow Lead Oxide. A raptorial ameba was isolated and a small mass of lead oxide placed in its path—473. As the ameba moved forward it turned toward the oxide—475, 476—showing that this material may be sensed at a distance of at least forty microns. After the ameba came into contact with the oxide a small pseudopod was thrown out posterior to it—479. The oxide was partially surrounded and the behavior suggested the first stage of ingestion, but the ameba moved on leaving the oxide finally behind—484. A new mass of oxide was then placed before the ameba—485. The ameba moved directly forward into contact with the oxide, and there was observed again what seemed like the initial stages of ingestion—487. The ameba then broke up into several pseudopods—488. A food cup was formed between the two pseudopods on the left, but nothing could be observed in it. It is probable that the presence of the lead oxide was the cause of the formation of the empty food cup. The ameba finally moved on leaving the oxide behind. A piece of globulin which was then laid before the ameba remained uningested perhaps because of the just previous disagreeable effect of the lead oxide. Several essentially similar instances are recorded in my previous papers cited above. A new mass of lead oxide was then placed in the path of another granular ameba—491. The ameba moved forward a short distance then turned to the right—492. The

ameba then broke up into four pseudopods, two of which were directed toward the oxide—494. The ameba moved off to the right without further reaction toward the oxide.

Lead oxide induces strongly positive behavior in raptorial amebas. Not only are the amebas induced to move toward this substance, but occasionally the initial stages of ingestion seem to be called forth by it. In this respect lead oxide stands on a level with, or above, some food substances such as zein or ovalbumin. In strong contrast to the behavior of raptorial amebas toward lead oxide is that of the granular amebas. In these, negative behavior is nearly always produced by this substance. Why there should be this difference is not clear. The solubility of this substance was not tested by me.

Among other insoluble substances that were used in these experiments is iron. This metal cannot be obtained perfectly pure and it also undergoes chemical action in the water. Particles of it were agitated by means of an electromagnet beneath the stage of the microscope, but the apparatus was rather crude and no definite results were obtained.

Reactions to Very Soluble Digestible Substances.

Substances belonging to this class, such as gelatine and tyrosin, are much less satisfactory to work with than those substances that are insoluble, or only very slightly soluble; for the stimuli proceeding from very soluble substances cannot be definitely localized, and the behavior often appears uncertain. The resulting behavior is consequently difficult to interpret. Notwithstanding these objections, experiments in which very soluble substances may be used are of value in order to learn in a general way what effect the degree of solubility may have on ameba.

Tyrosin.—The product used bore Merck's guarantee of purity. A mass of tyrosin thirty microns in diameter dissolves in water in about ten minutes. A grain of tyrosin was placed in the path of a granular ameba—6. The ameba turned to the right and moved on, avoiding the tyrosin. A new grain of tyrosin was then laid in the ameba's path and negative behavior again followed—10. A third grain of tyrosin was then presented—13—and the ameba moved on past it with apparent indifference.

(A grain of globulin which was next presented was, after some uncertainty in behavior, finally ingested in a food cup pointing upwards.) A few minutes after the globulin was ingested another grain of tyrosin was laid in the ameba's path—18. The ameba moved into contact with it and then ingested it while moving on over it. No period of rest ensued. Another grain of tyrosin was then laid a little to the right of the ameba's path, but before coming quite into contact with it, the ameba moved away to the left.

This series of experiments shows very well the effect of previous behavior upon a closely following reaction. First, two trials with tyrosin produced negative behavior. The third trial resulted in indifferent behavior, doubtless because it was the third time the test substance had been encountered. The ameba was a long time in eating a grain of globulin which was next presented, and at first the ameba reacted indifferently toward it. It is more than likely that the previous experience with tyrosin developed this condition of indifference in the ameba. But this condition was entirely overcome by the reactions involved in eating the globulin, for when the next grain of tyrosin was presented, it was ingested. Thus the effect of previous behavior influenced the ameba's succeeding reactions more than the nature of the stimuli received in these reactions. But the newly created tendency to positive behavior was of short duration, for when another grain of tyrosin was presented only mild positive behavior, followed by avoidance, was observed.

A tyrosin grain was placed in the path of another granular ameba—51. Very remarkable behavior followed. The ameba moved forward into contact with it and then proceeded to flow on over it. When the anterior end lay over the tyrosin, it formed itself into an inverted shallow cup over the tyrosin—55. But no sooner was the food cup formed and ready to close up over the tyrosin than the anterior end was lifted up, away from the tyrosin, and the middle and posterior regions of the ameba contracted. The effect was of course to remove the anterior end of the ameba from the dissolved or dissolving tyrosin. The food cup was completed however and persisted in the ameba for some time. The ameba moved away from the tyrosin for a short

distance but it soon came again within sensing range of the tyrosin grain—59. The ameba became aware of the center of diffusion of the tyrosin at a distance of about 125 microns. The ameba moved toward the tyrosin grain, then over it, then formed a food cup, and later withdrew, just as in the preceding trial. The tyrosin had gone completely into solution however when the ameba withdrew. Another tyrosin grain was then laid before the ameba—64. The ameba moved forward into contact with it and then repeated, substantially, the behavior observed in the two preceding tests.

After the ameba had withdrawn a short distance from the tyrosin, and had become more or less quiet, another ameba—71—came from the opposite direction and proceeded at once to form a typical food cup over the tyrosin grain. When the food cup was nearly completed over the tyrosin, it was suddenly extended to take in part of the original ameba—73. The tyrosin became imbedded in the protoplasm while the new ameba attempted to eat the other one—74. The attempt to capture the old ameba was soon given up as this ameba became active and moved out of reach of its would-be captor—75–77.

A new grain of tyrosin was laid in the path of the ameba that had been partially captured (the ameba shown in Fig. 70)—79. The ameba turned to the left avoiding the tyrosin, but later while passing by the tyrosin, a side pseudopod was sent out toward it—84, 85. This pseudopod moved over the tyrosin, swelled out and formed a food cup, and then withdrew from the tyrosin, just as in the previous experiments. A second attempt was made to move over the tyrosin—92, 93—but the pseudopod was retracted before the tyrosin was entirely covered. Then the vestige of the previous main pseudopod became active again and the ameba moved off. The food cup that was formed was completed. It remained undiminished in size for at least thirty minutes.

A grain of tyrosin was then laid to the right of the path of another granular ameba—98. The ameba sent out a pseudopod toward the tyrosin. After it came into contact with the tyrosin the ameba formed a food cup and ingested it. At the time of closing of the food cup the ameba loosened its hold on the sub-

stratum and rolled over, contracting antero-posteriorly at the same time—107. The effect of the tyrosin on this ameba was similar to that on the previous ameba, except that the tyrosin did not seem to act so intensely or so quickly on the latter ameba. A small grain of tyrosin—T_2, Fig. 109—was then laid on the ameba but no change of behavior was observed. Another grain of tyrosin was then laid in contact with the ameba at the anterior end—112. The ameba sent out a pseudopod at the anterior end, upward into the water, and out of contact with the tyrosin; but the weight of it became so great that the ameba keeled over and so was removed from contact with the tyrosin. Another grain of tyrosin was then placed in the path of the ameba—113. The ameba moved on over it with very slight change of behavior. Another grain of tyrosin was placed in the ameba's path—117—but after slight movement toward it the original direction of movement was resumed and the ameba moved on without further change of behavior toward the tyrosin. The tyrosin grain which this ameba ate remained in it for over two hours without apparent reduction in size.

Summary of Reactions toward Tyrosin.—The behavior toward tyrosin is anomalous; no other substance, so far as known, induces similar behavior. Although the ameba seems to be strongly attracted by the tyrosin, yet when the food cup is about to close up the ameba withdraws. The negative reaction is due apparently to too intense stimulation. In the case of one ameba the impulse to withdraw did not make itself felt until the tyrosin grain was eaten. The formation of the food cup also is peculiar. Only when stimulated with tyrosin is the food cup formed by a hollowing out of the under side of the pseudopod. No other substance has been observed to produce such behavior. In at least two instances the food cup was completed although the tyrosin grain was not in it. This indicates that the formation of food cups is somewhat of the nature of a reflex. The closure of the food cups in the circumstances described above could not be explained adequately by assuming that it was due to the direct effect of the tyrosin in solution upon the surface tension or other physical property of the ameba, as Rhumbler ('98, '10) contends. Tyrosin dissolves in the body of the ameba much more slowly

than in water. A grain of tyrosin that dissolves in water in ten minutes remains in an ameba for over two hours without appreciable diminution in size. This would seem to indicate that it is not sufficient for tyrosin to go into solution in order to be assimilated, but that it must be further broken down by digestive action; unless indeed the assimilation of dissolved tyrosin goes on very slowly. It is impossible to say at present why tyrosin remains undissolved for so long in the ameba's body. Negative behavior toward tyrosin is similar to that toward other substances. In one case negative behavior was changed to positive behavior by presenting the ameba with a grain of globulin. Before the globulin was ingested the tyrosin was avoided; after ingestion, a grain of tyrosin was eaten. This is a very good illustration of the possibility of habit formation in ameba. Tyrosin can be sensed at a distance of at least 125 microns. Although it is quite likely that the ameba reacts to tyrosin in solution in the experiments described above, yet the ameba invariably moves with great accuracy toward the center of diffusion, the tyrosin grain itself. The mere presence of molecules (or ions) in solution would therefore not explain the whole behavior. Some other factor must operate such as differences in the concentration of the molecules of tyrosin in solution, as would occur in the process of going into solution, for without some such additional factor the ameba would be unable to find the solid tyrosin.

Gelatin.—Knox's Sparkling (not acidulated) commercial gelatin was employed. Only a few tests were made, owing to the experimental difficulty of handling it. One experiment only is recorded in this paper.

A small piece of gelatin was placed to the right of the path of a granular ameba—1. A small pseudopod which was extended toward it came very nearly into contact with it, when the ameba turned to the right, avoiding the gelatin—4. A small pseudopod was then thrown out on the convex side of this pseudopod toward the gelatin, but it was retracted before it came quite into contact with the gelatin. The ameba finally moved on through the vestige of the previous main pseudopod.

Arrowroot Starch Paste.—This was made by boiling starch paste with water and allowing it to cool until a rather stiff gel

was formed. A small mass of this gel was placed in the path of an ameba—303. The ameba moved forward with a little uncertainty—304, 305. Presently, however, the ameba began to move in a concerted manner and when the main pseudopod was about one fourth past the starch paste, a pseudopod was sent out toward it, but the pseudopod was retracted before it came quite into contact with the paste. The forward movement of the ameba did not seem to be disturbed by the projection of the small side pod. The behavior of the ameba toward arrow-root starch paste is very similar to that toward gelatin.

Reactions to Substances in Solution.

Solutions of certain substances were allowed to run into very fine capillary glass tubes, after which one end of them was sealed hermetically with heat. The tubes were made a centimeter or more in length so that the substance at the open end of the tube would not be affected by the heat employed in sealing the other end. The external diameter of the tubes was about twenty-five microns, and the bore about fifteen microns, but there was some variation in the dimensions of the different tubes.

The solutions were placed in small tubes, for it is necessary to localize as definitely as possible the diffusion currents from the open end of the tubes in order to be certain of the meaning of the behavior which might be observed. But even with the employment of capillary tubes the results are more or less uncertain, unless the reactions are decided or repeated often; for, the solutions being in most cases colorless, the extent of their presence outside of the tubes can only be inferred.

Peptone.—From meat. Commercial, from Eimer and Amend. As is well known, this substance is very soluble in water and has a very strong agreeable smell and "taste." The taste is salty. The action of peptone resembles that of meat extracts upon the human senses.

A capillary tube filled with a dilute solution of peptone was placed in the path of a raptorial ameba—333. The ameba moved toward the open end of the tube and formed a large food cup before coming into contact with it, but the food cup finally closed in over the open end of the tube—334. The ameba

remained quiet for about six minutes, during which time the water and the peptone solution disappeared from the food cup. The ameba then started to move away. As the posterior end passed the tube opening another food cup was started over the tube but was not completed, and the ameba then soon moved away—335. But when the tube was again laid in the ameba's path—336—another food cup was formed over it. (A control tube containing culture fluid was then placed in the path of the ameba but only slight positive behavior resulted.) The peptone tube was then shifted—337. The ameba formed a complete food cup at a distance of about 100 microns from the end of the tube—337, 338—showing conclusively that substances in solution are capable of causing the formation of a normal food cup. The ameba then started to move away to the right—339—but presently changed its course, returned to the tube—340—and enclosed it again in a food cup—341. After remaining in this position for a minute and a half, the ameba moved off. (The control tube was then again laid before the ameba but no definite change in behavior was observed.)

A fresh tube containing dilute peptone solution was placed in the path of another raptorial ameba—342. The general result was negative behavior, due possibly to the commotion produced by placing the tube in position.

Another ameba in the same dish happened to come within range of the tube—346. A pseudopod was thrown out which moved directly toward the tube opening—347—but when it came within about 100 microns of the tube it turned to the right and moved on. The small pseudopod extended toward the tube from the convex side of the ameba as it turned to the right —349—indicates a slight tendency to positive reaction. The tube was then shifted—351. The ameba threw out a pseudopod which moved toward the peptone tube for some distance, then through a new pseudopod the ameba moved off to the right—352. While passing by the tube a side pseudopod was thrown out toward it—354—but it was soon partially retracted. Very soon it was again extended—356—and after it came within twenty microns of the opening of the tube, it was retracted, and the ameba moved directly away from the tube through a new

pseudopod. The final effect of the peptone in this case was to produce negative behavior, nevertheless the attractive qualities were quite strong.

In the path of another granular ameba was placed a new tube containing a dilute solution of peptone—358. A number of very small flagellates gathered near the open end of the tube. The ameba moved forward toward the tube until it came within thirty microns of it when a pseudopod was thrown out on the right—360—which appeared destined to become the main pseudopod, but the tendency toward positive reaction gained the upper hand again and a food cup was formed over the open end of the tube together with the flagellates, by a reactivation of the previous main pseudopod—362. Ten minutes after the formation of the food cup the ameba began to make efforts to move away. The protoplasmic current was reversed several times, but finally the ameba moved on, after having been in contact with the tube for eighteen minutes. It is impossible to tell whether the flagellates had any influence on the reactions in this experiment or not.

In the majority of cases peptone in dilute solution diffusing from a small capillary tube attracts amebas. In one ameba four food cups were induced, one being completely formed at 100 microns from the open end of the tube. There can be no doubt, then, that a solution of a chemical, such as peptone, even when entirely free from the presence of a solid, is an adequate stimulus to set off the feeding reaction. It is, nevertheless, interesting to note that in the other three cases of food cup formation the solid source, that is, the tube opening, was sought and enclosed as if the chemical in solution acted only as a guide to the solid object from which the chemical was diffusing.

Egg Albumin.—Capillary tubes were filled with a solution made from Eimer and Amend's crystallized egg albumin and filtered culture fluid. This solution diffuses much more slowly than peptone. The solutions were very dilute: one part of albumin to 200 parts water.

A tube of egg albumin was placed in the path of a granular ameba—369. The ameba moved forward into contact with the tube, though apparently without being attracted toward it.

After being in contact with the tube for a few minutes the ameba moved away from it about 100 microns, but soon returned again. The whole behavior is puzzling, and may have had no relation to the diffusing albumin.

A new tube of egg albumin was placed to the right of the path of a raptorial ameba—405. As the ameba passed the opening of the tube a pseudopod was thrown out on the side directly toward the tube—407—but it was retracted when about twenty microns from the tube—408. The ameba moved on without further reaction. The tube was then shifted—410—but it was definitely avoided by the ameba. When shifted again—415—it was again avoided. When shifted the third time—422—the behavior was indifferent.

Another tube of albumin solution was placed to the right of a raptorial ameba—426. A pseudopod which was thrown out on the right moved directly into contact with the open end of the tube—432. From this pseudopod another was sent out which moved along the side of the tube, and through it the ameba moved off—433, 434. There was no attempt made to form a food cup at any time.

In the path of another raptorial ameba was placed a fresh tube of albumin—436. This ameba was very strongly attracted by the albumin, for five food cups (451, 452, 455, 466, 469) were formed over the tube opening during the forty-one minutes the ameba remained in contact with the open end of the tube. Only the first food cup was completely closed; the others were only partially formed. This is the only certain case where a food cup was formed over the albumin because of stimulation proceeding from the albumin.

To summarize: The majority of amebas experimented upon reacted with indifference, or negatively, to the egg albumin in capillary tubes. Only one reacted decidedly positively, but this one formed five food cups in succession over the open end of the tube. There can be no doubt then of the efficiency of albumin as a stimulator for setting off the feeding process; nevertheless the stimulus seems to be weak as compared with that from peptone.

Tyrosin.—A tube filled with weak tyrosin solution was placed in the path of a raptorial ameba—398. A pair of side pseudopods

were formed near the tip of the main pseudopod, the right one of which moved toward the tyrosin tube; but this pseudopod was soon arrested and retracted while the main one enlarged rapidly for a short time when it also was retracted. The pseudopod on the left then became the main one through which the ameba moved away after ingesting a flagellate—402–404. The tube was then shifted, but the resulting behavior was indifferent.

Carmine.—A capillary tube filled with a solution (not a suspension) of carmine was placed in the path of a raptorial ameba—383. The ameba moved toward the tube a short distance—384—then reversed streaming and moved away. A new tube of carmine solution was placed before another raptorial ameba—386. The ameba was disturbed in its movements but finally sent out a pseudopod toward the carmine tube—388. But when it came within about 100 microns of the tube it broke up into several pseudopods—389—and finally the ameba moved away to the left. The tube was then shifted—393. The ameba moved toward the tube a short distance, then stopped and sent out a pseudopod on either side—395. The one on the left enlarged first, but when it came near the tube it was retracted and the one on the right enlarged and carried the ameba away—396. The tube was then laid before the advancing pseudopod—397. This pseudopod was at once arrested and the pseudopod just previously retracted became active and carried the ameba away.

These experiments indicate that solid particles of carmine are very much stronger in their stimulating power than solutions of carmine. Carmine grains nearly always produce positive behavior which results in contact, while solutions induce only negative reactions. It is at present impossible to tell why there should be such a difference, for the chemical nature of the substance is presumably the same in both cases. Better technic doubtless would do much to solve this difficulty.

A number of experiments were also performed with sodium chloride in small tubes, but the results were almost exactly like those in which culture fluid was used for filling the tubes. Here also better technic would probably give interesting results.

Summary.

1. Ameba senses small particles of insoluble substances such as carbon, glass, silicic acid, etc., at a distance of from 60 to 100 microns. The reaction is nearly always positive and it consists in the turning of the main pseudopod toward the test object or in the projection of a pseudopod on the side of the main one toward the object. The side pseudopod may or may not become the main pseudopod. After the test object is touched the ameba usually moves on without further change in behavior.

2. Although the facts are clear, the explanation of reaction at a distance to insoluble substances is lacking. Surface action of some sort or the reflection of light from the test object are possible factors. But whatever the explanation, the ability of an eyeless animal like ameba to sense insoluble objects at a distance is without parallel among organisms and it is consequently a phenomenon of fundamental importance in sense physiology.

3. Ameba reacts positively to tyrosin. The behavior toward small grains of tyrosin is very peculiar. The ameba moves toward the tyrosin as if strongly attracted and begins the formation of a food cup. The stimuli then seem to become so intense that they produce a negative effect in the reactions, and the ameba withdraws from the tyrosin grain. Tyrosin grains are however occasionally eaten.

4. The reactions toward tyrosin refute completely the contention (if it still needs refutation) that the eating process in ameba is purely a surface action effect of the stimulating object.

5. Both egg albumin and peptone in weak solution diffusing from a capillary tube cause the formation of food cups. In nearly all cases the food cups were formed over the open end of the tube as the solid source of the diffusing albumin or peptone; but in one or two cases a food cup was formed at a distance from the tube. Peptone stimulates the ameba much more readily than albumin.

6. Solutions of carmine and tyrosin in capillary tubes do not readily attract amebas. This is in strong contrast to the behavior toward grains of these substances, which is almost always positive. The difference in behavior as recorded is perhaps due to faulty technic in handling the capillary tubes.

7. The experiments indicate that ameba is sensible to at least two kinds of stimulation: that from insoluble solids such as carbon and glass, and that from substances in solution, such as peptone or albumin. It seems hardly possible that carbon and glass, at a distance from the ameba, produce the same disturbance on the surface of the ameba, such as a change in surface tension, as contact with dissolved peptone. A definite conclusion however awaits further work.

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EXPLANATION OF PLATES.

The figures are camera lucida drawings taken from the laboratory notes without alterations. The camera lucida was attached to the right hand tube of a long arm Zeiss binocular microscope. Eyepiece 4 and objective a_3 were used, giving a magnification of 65 diameters. A scale by means of which the size of the amebas and of test objects can be estimated is shown on Plate 7.

The figures are numbered serially from 1 on for reference. An x following a number, as 9x, indicates the end of the experiment illustrated by Figs. 6 to 9x inclusive. A new experiment starts with Fig. 10 and ends with Fig. 12x, and so on. If a number is followed by xx, it means that the next experiment was performed upon a different ameba. Thus Figs. 6 to 27xx represent the results of several experiments upon the same ameba. With Fig. 28 a new ameba was employed, and so on. The order in which the figures were drawn is represented by the serial numbers for all the figures in any one experiment, and in nearly every case for all the experiments performed upon any one ameba.

The time of the beginning and the end of each experiment is given in hours and minutes. In many cases the time of drawing of each figure is also given, and where it is not given it may easily be computed.

The arrows show the direction of active protoplasmic streaming. The arrows in the last figure of each experiment denote the direction the ameba took in moving away from the test object.

The test objects are labelled in abbreviated form. See table of abbreviations below. For quick and correct reference the test objects are connected with the proper ameba by leader lines. These lines have no other significance.

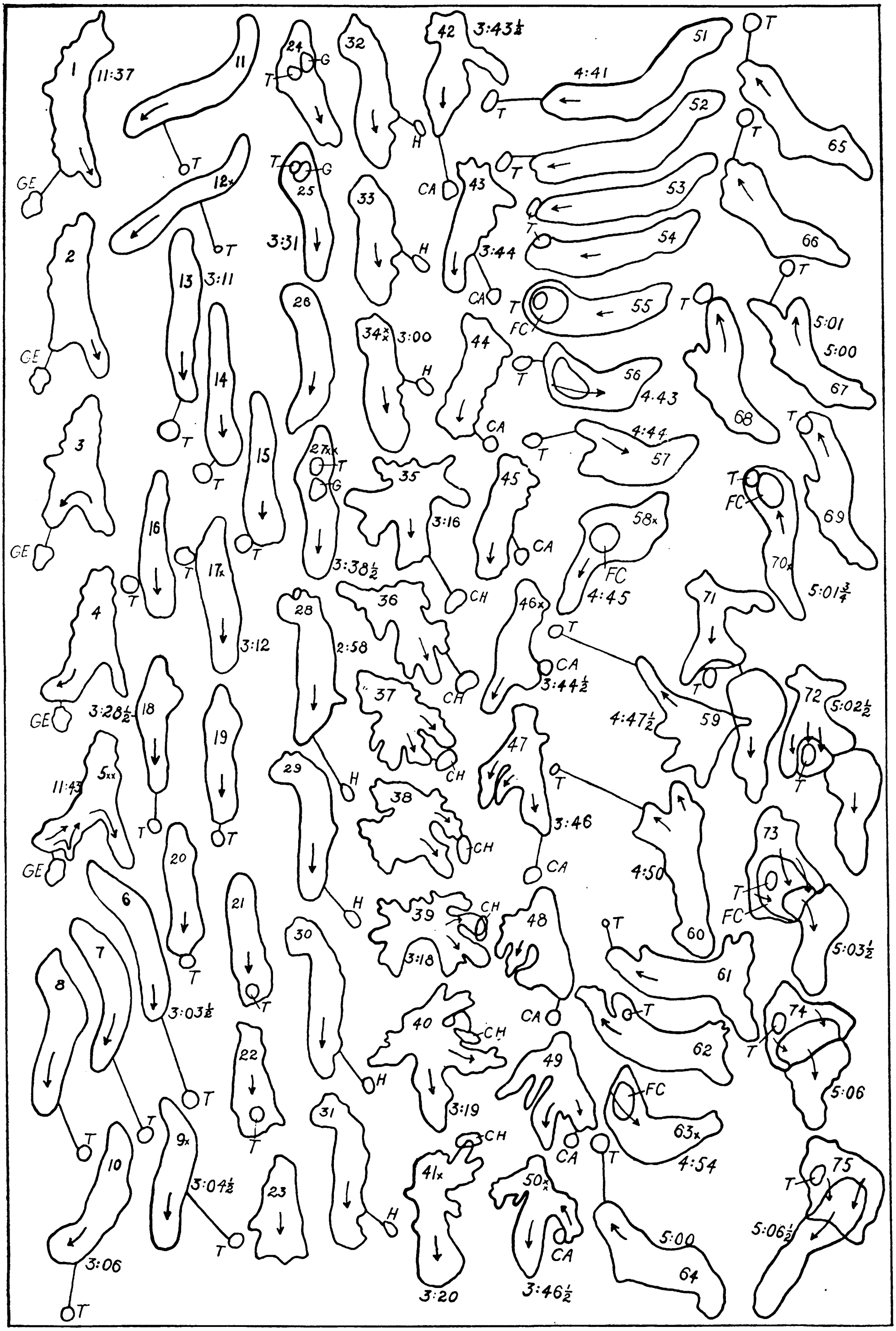
All the work was done facing a north window. All the figures were drawn in the same position in the laboratory and on the plates. The top of each plate therefore points toward the north. This is worth noting from the point of view of the possible influence of light on the behavior of ameba.

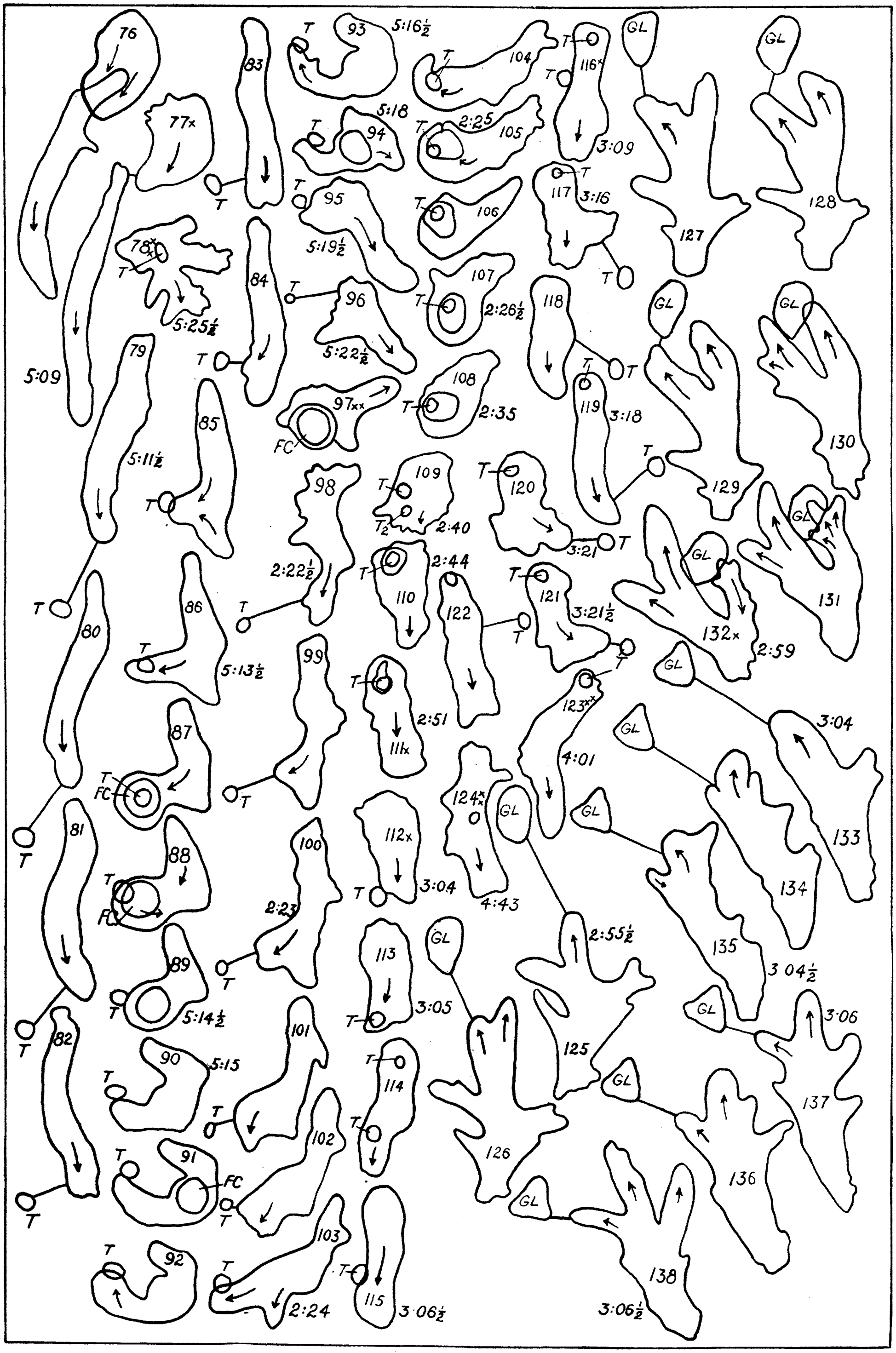
It will be noted that there are slight differences in the size and shape of the same test object as drawn in the figures of any single experiment, even if the object was not rolled around by the ameba. The explanation for this difference lies in the speed with which the drawings had to be made in order to catch important items of behavior. As a rule the parts of the ameba lying nearest the test object received the most careful attention and were drawn first; the posterior parts of the ameba and the test object were drawn last.

For detailed explanation of figures see text.

Table of Abbreviations.

AR, arrowroot starch grains.	*GE*, gelatin.
ARP, arrowroot starch paste.	*GL*, glass.
C, carmine solution.	*GR*, graphite.
CA, carbon.	*H*, hematin.
CH, cholesterin.	*IN*, indigotin.
E, egg albumin solution.	*P*, peptone.
FC, food cup.	*PB*, lead oxide.
FL, flagellates.	*S*, silicic acid.
G, globulin.	*T*, tyrosin.





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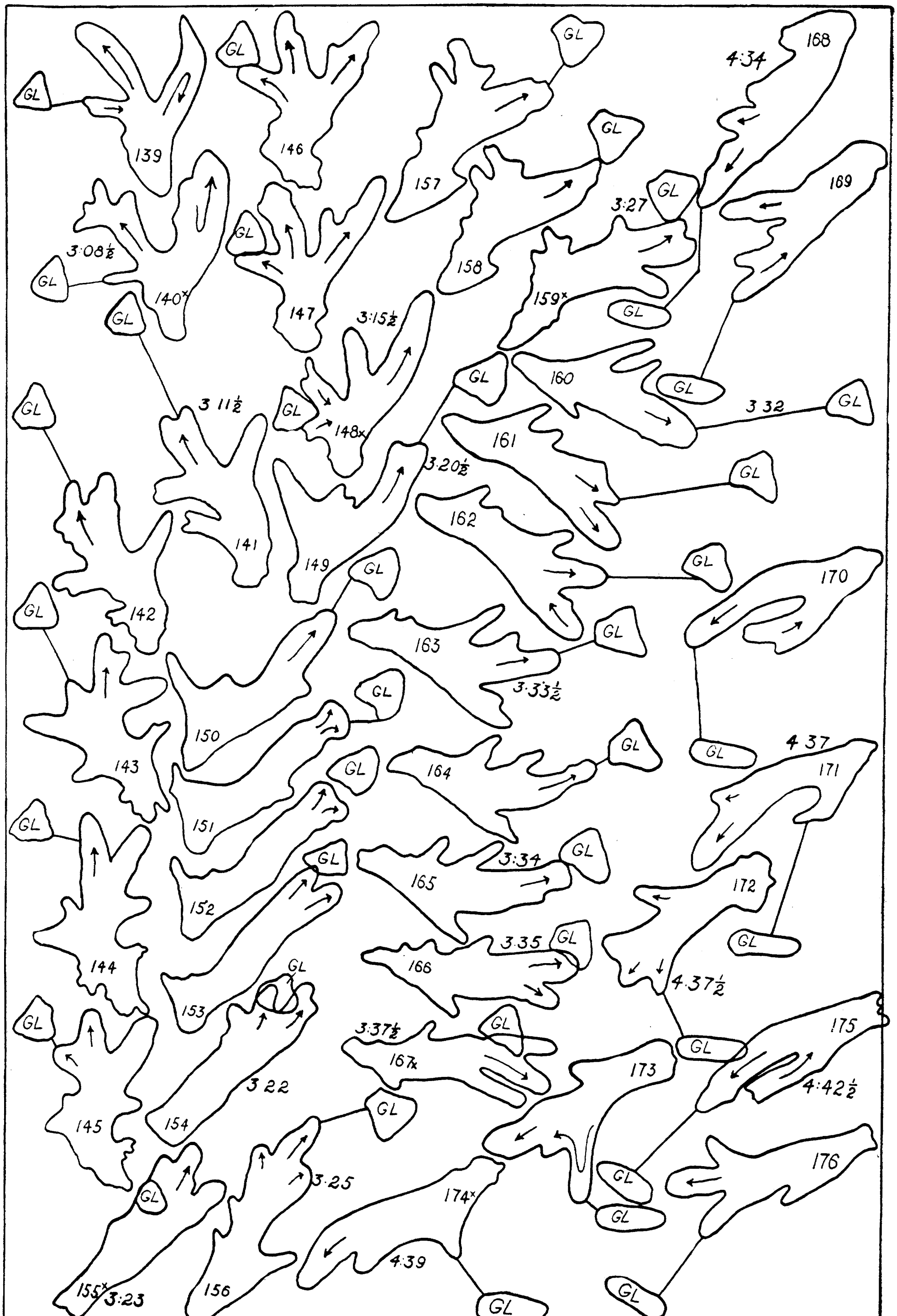
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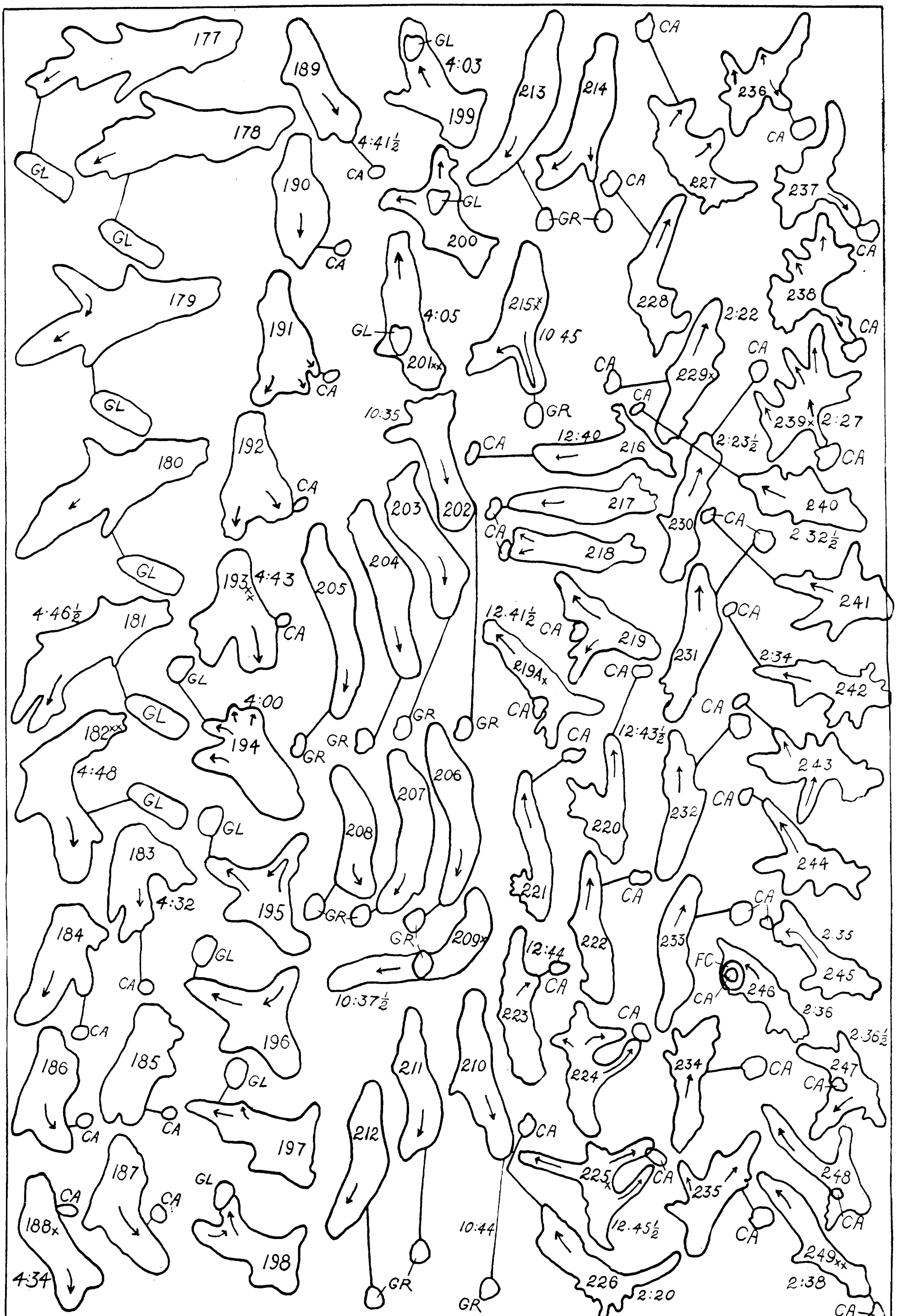

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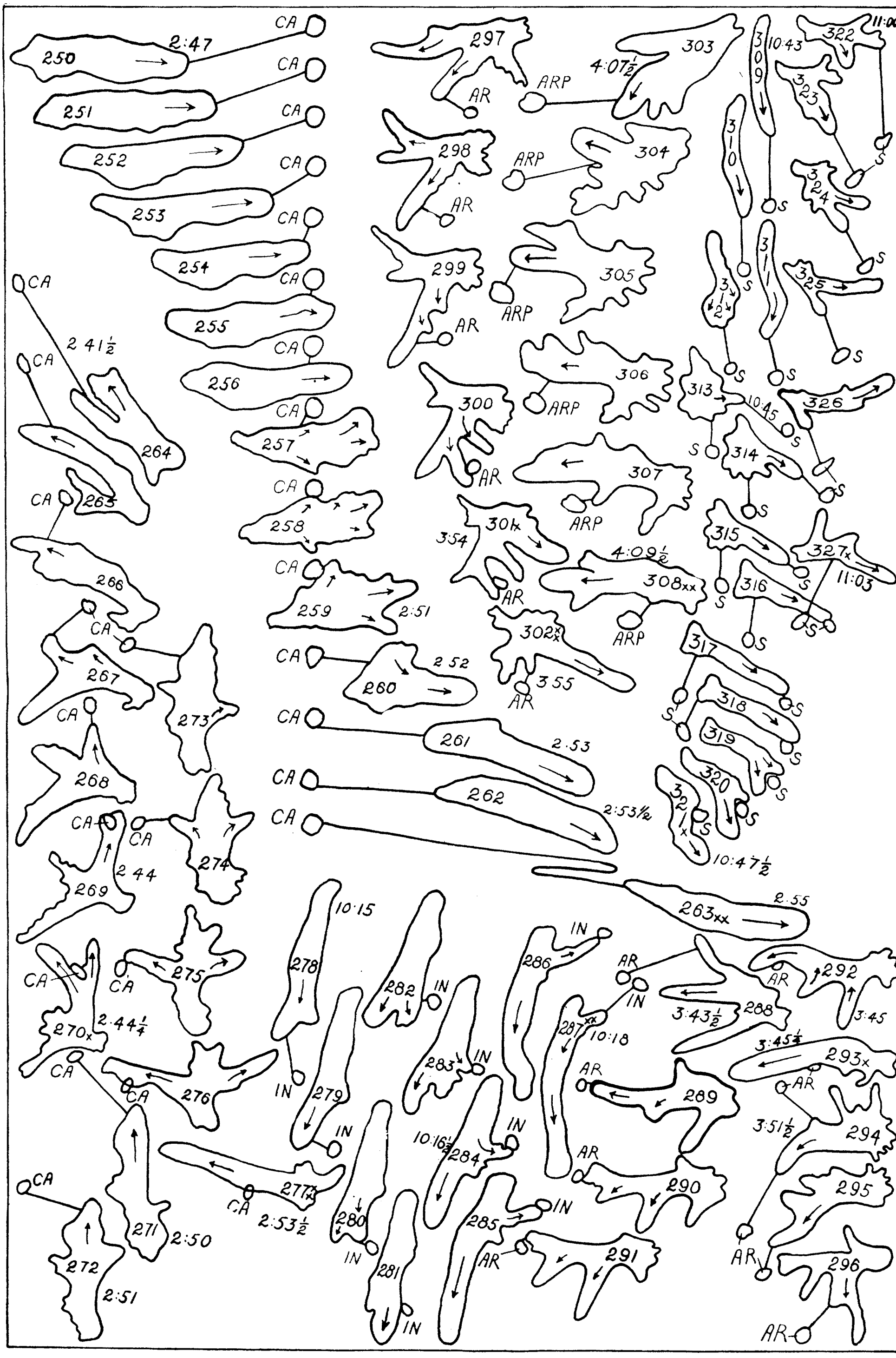

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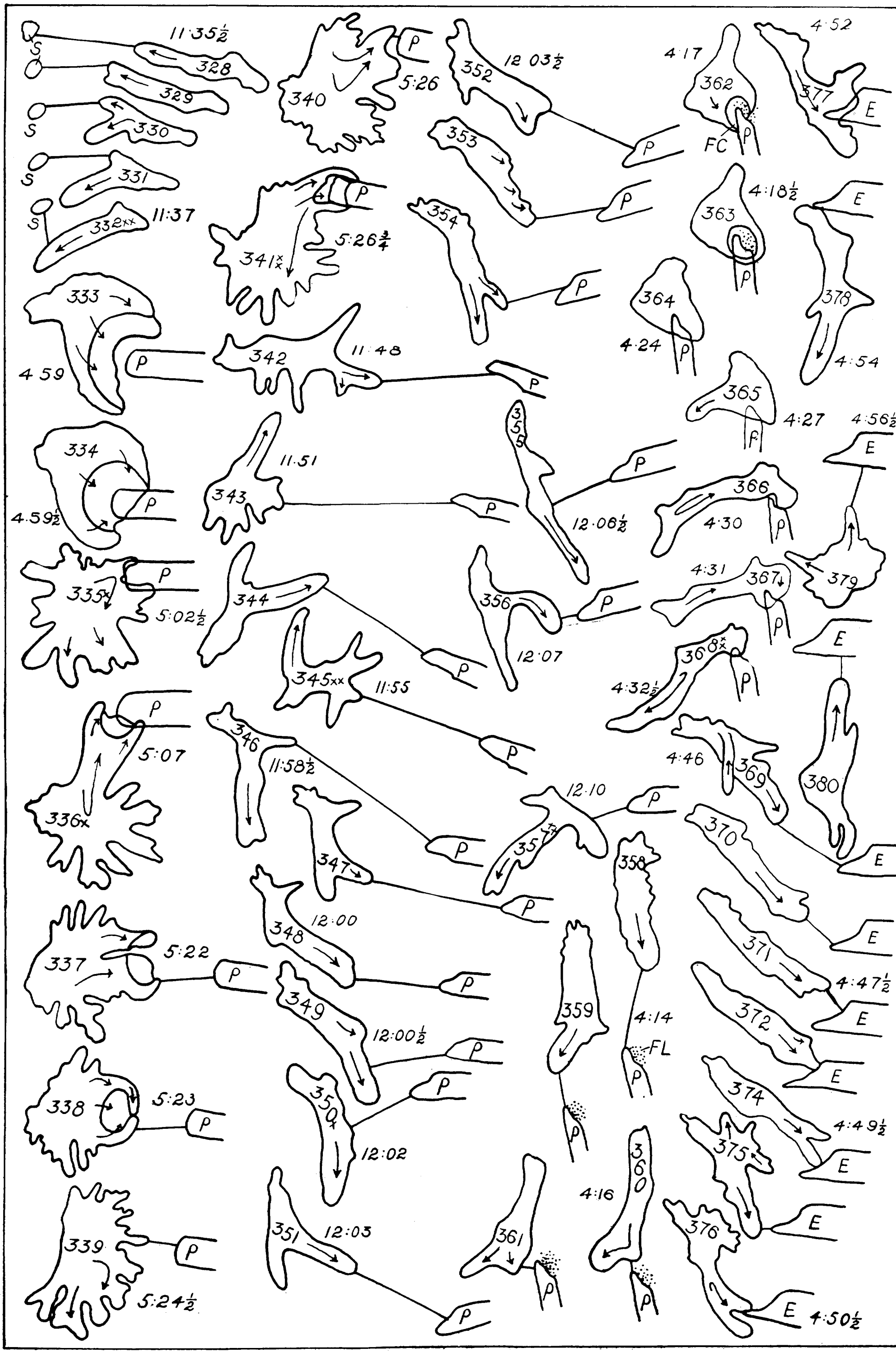

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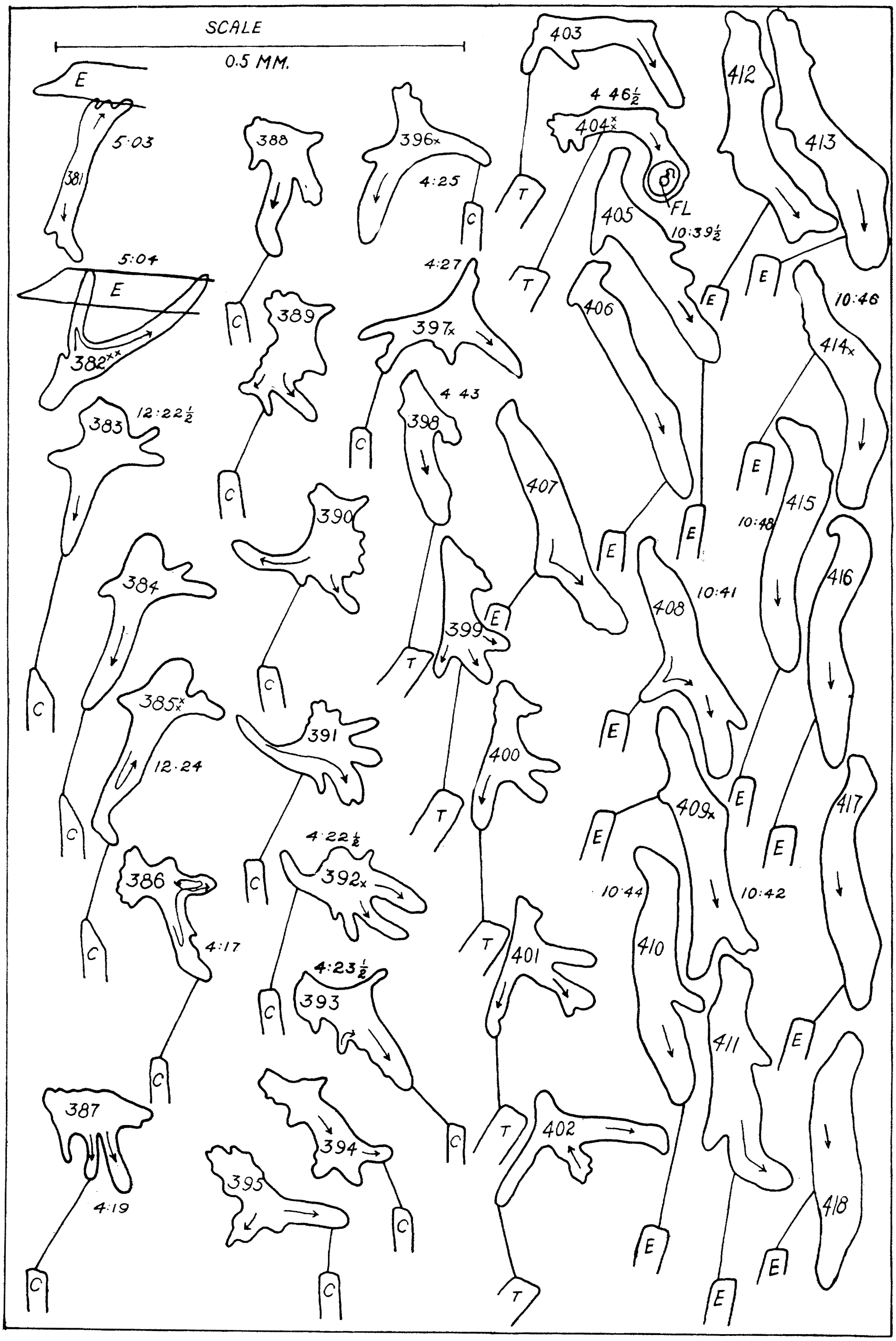

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